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REVIEW

Recent Progress in Comparative Neuroimmunology

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INTRODUCTION

During the past decade, broadly based comparative studies on cell-mediated immunoregulatory processes have revealed remarkable parallelisms between vertebrates and higher invertebrates (see Scharrer [1]). One distinctive feature these two groups have in common is the use of the same or closely related chemical messenger substances in internal immunoregulatory processes as well as the bidirectional exchange of information between the immune system and the neuroendocrine system [2]. Neuropeptides, formerly considered to be confined to the neuroendocrine apparatus, are now known to be produced by and released from immune cells. By the same token, cytokines, i.e., regulatory molecules characteristic of the immune system, have been shown to be manufactured also by neural elements, including astrocytes. Information on the identities of these signal molecules in invertebrates as well as vertebrates has been obtained by use of biochemical and immunocytochemical methods, and by *in vitro* and *in vivo* tests on the effects of various exogenous analogs.

The immunoactive cells of the invertebrates that have been primarily used in these studies, the mollusc *Mytilus edulis* and the insect *Leucophaea maderae*, are subpopulations of the animals' hemocytes. They share a number of properties with cells of the mammalian granulocyte-monocyte-macrophage lineage. Evidence for the existence of different subsets of invertebrate immunocytes was obtained by the observation of differences between the modulatory effects of opioid peptides and those of certain other ligands [3]. Like their counterparts in vertebrates, these cells respond to immunomodulatory stimuli by characteristic conformational changes indicative of cellular activity. For these light microscopic observations immunocytes were incubated together with various ligands at 37°C for human, and at 23°C for *Mytilus* and *Leucophaea*. Either a Zeiss Axiophot Microscope and the Zeiss Videoplan/Vidas Image Analysis System [4] or the system designed by American Innovision, Inc. were used. Changes in cellular conformation were determined by measurements of cellular area and perimeter, mathematically expressed by use of the Zeiss Form-Factor (FF) forumula [4]. Additional factors, introduced by Schön *et al.* [5], permitted a distinction between types of cellular change, i.e., those obtained in different animal species by the same signal molecules and those effected by different substances in the same species.

Neuropeptides

The search for multiple functional roles of neuropeptides in signaling within as well as outside of the nervous system has taken a number of unexpected turns since their discovery in special neurosecretory neurons some 70 years ago. Currently available evidence indicates that members of this large family of compounds, especially opioid peptides, may have dose-dependent stimulatory as well as suppressive effects on various immunoregulatory processes. The substances acting in these capacities are furnished by immunoactive cells as well as cells of the neuroendo-

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crine system.

The capacity of invertebrate immunocytes to produce and release a Met-enkephalin-like material was demonstrated by high pressure liquid chromatogaphy and radioimmunoassay. This material was found to be present in cell-free hemolymph as well as hemocytes of *Mytilus* [4]. Evidence for its presence in *Leucophaea* hemolymph is more tentative (see Scharrer *et al.* [6]). Met- and Leu-enkephalin was also identified in the pedal ganglia of *Mytilus* [7].

Immunocytochemical methods have revealed substances antigenically related to Met-enkephalin and numerous other neuropeptides in the neuroendocrine system of *Leucophaea* and other insects (see Scharrer *et al.* [6]).

1. Stimulatory activities

Immunocytes of invertebrates and vertebrates respond to stimulation with appropriate concentrations of opioid and other peptides by increased adherence to albumin-coated slides and the formation of clumps. Prior to becoming mobile, these cells flatten and change their structure from rounded to ameboid (*Mytilus*) or elongated (*Leucophaea*, human). The addition of the opioid antagonist naloxone to the preparation, concurrent with the cells' exposure to exogenous opioids, blocks their activation. Activated immunocytes of *Mytilus* have been observed to move in the direction of cell clusters of the same type [4].

Moreover, *in vivo* experiments with this mollusc or mammalian laboratory animals showed that chemotactic movements of immunoactive cells are guided by concentration gradients of certain specific recognition factors. Thus an immune-type response, experimentally induced in *Mytilus* by the severance of a nerve, resulted in the migration of immunocytes and their gradual accumulation in the lesioned area. After the injection of DAMA (D-Ala^2-Met^5-enkephalin) into an intact animal, but not that of a number of control substance, the cells accumulated at the site of injection [4].

An important feature observed by Stefano *et al.* [8] was that the stimulatory effect of Met-enkephalin on cellular conformation and locomotor activity is significantly higher than that of related neuropeptides or nonpeptide substances. This information was initially obtained by tests with the synthetic analog of Met-enkephalin, DAMA, which is resistant to the effect of the naturally occurring neutral endopeptidase 24.11 (NEP). The same high potency values (10^{-11} M) were accomplished by the administration of Met-enkephalin or of the heptapeptide Met-enkephalin-Arg^6-Phe^7 with the addition of the special enzyme inhibitor phosphoramidon [9–11]. In eight additional drugs examined, including the closely related Leu-enkephalin, the required concentration for comparable stimulatory effects was in the area of 10^{-9} M. Beta-endorphin was active at 10^{-10} M [8].

The search for stereoselective mechanisms mediating the immunostimulatory effects of opioids and nonopioid substances has provided pharmacological and binding evidence for the presence of receptors on immunoresponsive cells. The results of Scatchard analysis indicate that these binding sites on human granulocytes and *Mytilus* immunocytes are monophasic, stereospecific and of high efficiency. In both vertebtrate and invertebrate tests the complexity of ligand-receptor binding observed turned out to be greater than anticipated. Multiple opioid receptors, including delta-, mu-, kappa-, and presumably epsilon-receptors are preferentially used by different messenger molecules performing selective functions [8].

The high potency of Met-enkephalin demonstrated in *in vitro* tests with *Mytilus* and human granulocytes suggests that it plays a distinctive role in immunoregulation. On the basis of mammalian *in vivo* experiments and of preliminary clinical tests, Jancović *et al.* [12] called attention to the potential value of Met-enkephalin in the treatment of certain immune diseases. The proposal made by Stefano *et al.* [8] that, in its immunoregulatory role, Met-enkephalin interacts with a special subtype of opioid receptor ($delta_2$) has been substantiated by recent experiments with the equally potent opioid (D-Ala^2) deltorphin I [13]. Preincubation of human granulocyte membranes with DALCE (a select detla-opioid antagonist) revealed a binding capacity of this opioid, as well as that of DAMA, distinctly different from that of other opioids.

In comparing the modulatory effects of various

opioids on certain growth processes, Zagon *et al.* [14] likewise found Met-enkephalin to be more potent than the rest. This pentapeptide had a strong inhibitory effect on the growth of neuroblastoma cells in tissue culture. The special receptor proposed to be selectively involved was found in abundance in the cerebellum of infants and in a human brain tumor, but not in the cerebellum of normal adults.

2. Immunosuppressive effects

In contrast to most of the neuropeptides tested thus far two, derived from pro-opiomelanocortin, i.e., ACTH and MSH, are immunosuppressive. When tested in appropriate concentrations, they have been shown to inactivate immune cells that are either spontaneously active (5–10% in untreated preparations) or have been experimentally activated by other signal molecules known to have a stimulatory effect [11]. Part of this activity of ACTH seems to be indirect, i.e., by its conversion to MSH under the influence of the special endopeptidase (NEP) mentioned earlier. These observations have been made in experiments with vertebrate and invertebrate immune cells.

Information on additional immunoregulatory effects brought about by various neuropeptides is primarily based on mammalian studies. Such effects include antibody production, histamine release from mast cells, cytokine activity, vasodilation in inflammatory area, proliferation of lymphocytes, and phagocytotic activity.

Cytokines

In the search for basic immunoregulatory principles shared by invertebrates and vertebrates, the results of pilot studies on the presence and activity of cytokines in molluscs and echinoderms promise to be as rewarding as those on neuropeptides. The hemolymph of *Mytilus* was found to contain immunoactive interleukins (IL-1 and IL-6) as well as tumor necrosis factor (TNF-α). Its immunocytes respond to the respective mammalian cytokines *in vitro* and *in vivo* in a manner comparable to that of human granulocytes [15]. Like in mammals, IL-1 appears to participate in the internal regulation of the immune system of *Mytilus* in part indirectly, i.e., by its stimulatory effect on the formation of TNF. Moreover, IL-1 and TNF were demonstrated to be present in the hemolymph of *Leucophaea* (T. K. Hughes, Jr., Personal communication).

A molecule with the biochemical and biological characteristics of mammalian interleukin 1 has also been reported to be produced by an echinoderm, *Asterias forbesi* [16].

Mode of operation of immunoregulatory substances

Neuropeptides, cytokines and additional factors (including special enzymes) interact to form an efficient immunoregulatory network. Depending on their concentration and additional determining factors present, certain messenger molecules may be either immunostimulatory or -inhibitory. In addition to its immunosuppressive function, mentioned earlier, ACTH may stimulate the activity of B-lymphocytes and the phagocytotic capacity of molluscan immunocytes [17]. Its own production, as well as that of endorphins, by immune cells can be induced by another neuropeptide, corticotropin releasing hormone [18]. Conversely, the synthetic opioid DAMA has been shown to stimulate the formation of an IL-1-like molecule in human and *Mytilus* immune cells [11].

One way in which the degree of cellular activation is kept within appropriate limits is by enzymatic degradation of some of the signal molecules involved [10]. The observation that the local concentration of Met-enkephalin (and of the related heptapeptide Met-enkephalin-Arg^6-Phe^7) is downregulated by NEP also applies to the invertebrate *Mytilus* [11]. Tests with two major compounds resulting from the hydrolysis of the heptapeptide showed that they antagonize its effect, presumably by competing for part of the same receptor sites as those used by the heptapeptide. A balancing mechanism is thus provided by the enzymatic generation of antagonistic fragments from the agonist's molecule.

Interference with the normal operation of the immunoregulatory network may occur in various ways and may have important biomedical consequences. In schistosomiasis, immunosuppression, attributable to the release by an invertebrate parasite of immunosuppressive signal molecules resembling those of the host, appears to influence

the course of the disease [19]. Similarly, the survival of the human immunodeficiency virus in patients seems to be supported by its ability to stimulate the production of ACTH by the host [20].

An involvement of neuropeptides in the response to stressful conditions has been demonstrated not only in vertebrates, but also in the mollusc *Mytilus* [21]. The animal's immune/defense system can be alerted, for example, by mechanical interference with the valves of the in-current syphon. This leads to a significant rise in the number of "activated" immunocytes, presumed to be due to the release of an endogenous opioid-like material from the brain.

In conclusion, the rewards gained from a broadly based comparative approach to the study of neuroimmunological phenomena reach beyond the elucidation of commonalities between vertebrates and invertebrates. They provide information on the evolutionary history of a basic biological phenomenon and open new vistas in its exploration.

REFERENCES

1 Scharrer, B. (1991) Adv. Neuroimmunol., **1**: 1–6.

2 Blalock, J. E. (1989) Physiol. Rev., **69**: 1–32.

3 Hughes, T. K., Jr., Smith, E. M., Barnett, J. A., Charles, R., and Stefano, G. B. (1991) Cell Tissue Res., **264**: 317–320.

4 Stefano, G. B., Leung, M. K., Zhao, X. and Scharrer, B. (1989) Proc. Natl. Acad. Sci. USA, **86**: 626–630.

5 Schön, J. C., Torre-Bueno, J. and Stefano, G. B. (1991) Adv. Neuroimmunol., **1**: 252–259.

6 Scharrer, B., Stefano, G. B. and Leung, M. K. (1988) Cell. Mol. Neurobiol., **8**: 269–284.

7 Leung, M. and Stefano, G. B. (1984) Proc. Natl. Acad. Sci. USA, **81**: 955–958.

8 Stefano, G. B., Cadet, P. and Scharrer, B. (1989) Proc. Natl. Acad. Sci. USA, **86**: 6307–6311.

9 Shipp, M. A., Stefano, G. B., D'Adamio, L., Switzer, S. N., Howard, F. D., Sinisterra, J., Scharrer, B. and Reinherz, E. (1990) Nature, **347**: 394–396.

10 Shipp, M. A., Stefano, G. B., Switzer, S. N., Griffin, J. D. and Reinherz, E. L. (1991) Blood, **78**: 1834–1841.

11 Stefano, G. B., Shipp, M. A. and Scharrer, B. (1991) J. Neuroimmunol., **31**: 97–103.

12 Janković, B. D., Lević, Z., Nikolić, J. and Strojisavijević, N. (1989) Int. J. Neurosci., **48**: 155.

13 Stefano, G. B., Melchiorri, P., Negri, L., Hughes, T. K. and Scharrer, B. (1992) Proc. Natl. Acad. Sci. USA (in press).

14 Zagon, I. S. and McLaughlin, P. J., (1989) Brain Res., **480**: 16–28.

15 Hughes, T. K., Jr., Smith, E. M., Barnett, J. A., Charles, R. and Stefano, G. B. (1991) Dev. Comp. Immunol., **15**: 117–122.

16 Beck, G. and Habicht, G. (1991) Mol. Immunol., **28**: 57–584.

17 Ottaviani, E., Petraplia, F., Montagnani, G., Cossarizza, A., and Monti, D. (1990) Regul. Peptides, **27**: 1–9.

18 Simith, E. M., Morrill, A. C., W. J. III, and Blalock, J. E. (1986) Nature, **321**: 881–882.

19 Duvaux-Miret, O., Stefano, G. B., Smith, E. M. Dissous, C. and Capron, A. (1992) Proc. Natl. Acad. Sci. USA, **89**: 778–781.

20 Smith, E. M., Hashemi, F. and Hughes, T. K., Jr. (1991) FASEB J., **5**: 1486

21 Stefano, G. B., Cadet, P., Dokun, A. and Scharrer, B. (1990) Brain, Behav., Immun., **4**: 323–329.